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Methods of Analysis of Bovine Excreta for Gross Energy 1/

W. P. Flatt, H. F. Lawson, D. R. Gilliam, H. F. Righter,
D. R. Waldo, C. E. Coppock and P. J. VanSoest
Dairy Cattle Research Branch, Animal Husbandry
Research Division, Beltsville, Maryland

The losses of nitrogen, carbon and energy containing volatile compounds during the preparation and storage of bovine excreta have been the concern of research workers for a number of years. Unaccountable losses of these materials could lead to erroneous conclusions regarding carbon, nitrogen and energy balances. Suitable analytical procedures for nitrogen and carbon in fresh feces and urine are available, so these losses may be avoided or corrections applied. However, the precise determination of the gross energy of high moisture materials is more difficult.

Colovos et al. (1957) proposed a method of burning wet feces and silage by the use of 95% ethanol as a primer to facilitate combustion. Using this procedure, he demonstrated appreciable losses of energy when bovine feces (4.1-21.0%) and silage (8.3-21.0%) were dried. Losses of this magnitude could seriously affect any balance experiment, so gross energy determinations in this laboratory have been routinely conducted on both the wet material and dried pulverized samples. In the initial study reported by Flatt (1957) ethanol was used as the primer, and losses due to drying bovine feces amounted to only 3.27± 3.08% of the gross energy. The precision obtained with the wet combustion method was too low for detecting small losses, with the standard error of duplicate analyses being 1.86% for fresh feces, 1.34% for wet canned feces (Jacobson et al. 1959), and 0.42% for dried pulverized feces. Investigations of other compounds for use as primers followed, and Coppock and VanSoest (1959) reported that butyl cellosolve and dimethyl formamide were effective as primers for silage and feces, but that no primer was found which was suitable for burning fresh urine. The unfavorable primer to sample ratio (20:1 or greater) necessary to obtain combustion resulted in a loss of precision.

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Dimethyl formamide was used by Fenner and Archibald (1959) and Fenner (1959) in a study on the energy determination of 8 samples of fresh and dried cow feces. The difference between the energy content of freeze-dried, oven-dried, and fresh feces calculated to a comparable basis was less than 2%. The average variation of triplicate determinations of fresh feces using dimethyl formamide as the primer was 1.02% as compared to 0.19% for duplicate determinations on freeze-dried feces and 0.16% for duplicate determinations on oven-dried feces.

Bratzler and Swift (1959) conducted a study using 12 samples of cow feces to compare ethyl alcohol and benzoic acid as primers. The standard error of duplicates calculated from their data was 5.6% for 95% ethyl alcohol, 3.05% for benzoic acid and 0.31% for oven-air dried feces. They detected no loss of gross energy due to drying the feces, but there was a loss of 5.15± 1.89% of nitrogen, and an average loss of 727 mg. of carbon dioxide per 100 g. of fresh feces, which was attributed to having been present in the fresh feces as carbon dioxide rather than as combustible compounds.

The series of studies reported in this paper were conducted to determine the magnitude of errors inherent in the wet combustion of feces, the suitability of various materials as primers, and the possible losses of energy due to drying feces at different temperatures. Samples of feces collected from cows receiving only hay, pelleted hay, only grain, and mixed rations and steers consuming pelleted mixed rations were used in these studies. Results of 518 analyses of wet feces, 102 analyses of dried pulverized feces and 119 primers using a Parr adiabatic oxygen bomb calorimeter are summarized in Tables 2 through 8.

One of the major limitations of a primer is that errors are magnified. Any error in the addition of the primer or in the caloric value assigned to it is attributed to the sample, so it is necessary to use extreme caution in the measuring and mixing of the primer with the sample. Volatile losses can be serious, so in the selection of a primer, low volatility is desirable. Table 1 illustrates the extent of volatile losses of several compounds which have been used as primers. Samples of 1 gram each were weighed into open glass weighing dishes and weighed at one minute intervals for five minutes. The materials with a boiling point of 170° C. or higher did not show a detectable loss, so they could be weighed into the combustion capsule or pipetted. Ethanol was so highly volatile that only pipetting would be rapid enough to prevent significant losses.

Another factor to be considered is the stability of the primer. During the course of the experiments using dimethyl formamide as the primer, it became evident that the caloric value gradually declined due to its hygroscopic nature. Precautions to prevent this change included keeping the stock solution tightly sealed and the working bottle in a desiccator. Table 2 contains the data on the caloric values of the primers which were used, and it may be noted that the standard deviation of the dimethyl formamide was 23.3 cal./g for 73 analyses, while the standard error

of duplicates analyzed on the same day was only 9.89 cal./g. During routine work duplicate analyses of the primer were run with each series of fecal samples.

A pilot study of different primers and methods of determining the gross energy of feces was conducted using a sample of feces collected from a cow receiving only hay. In order to eliminate inaccuracies due to dry matter determinations from these comparisons, a series of 36 samples (1.5-2.5 grams each) were weighed into Parr combustion capsules from one sample of feces. The primers used and results obtained are presented in Table 3. The standard error of duplicate determinations was calculated from data where no incomplete combustions occurred. All values are expressed on the wet basis, and the caloric value of the dried pulverized feces was calculated to a wet basis for comparison. On the basis of this trial, further studies were planned in which dimethyl formamide and butyl cellosolve (ethylene glycol monobutyl ether) were used as primers. Table 4 illustrates the results of 148 analyses of feces collected from steers receiving pelleted mixed rations. It is apparent that while the standard error of duplicates is low (6-14 cal./gram) the relative per cent error is greater for all samples weighed on a wet basis. Another factor which may be noted is the comparatively large number of incomplete combustions and widely discrepant values which resulted from accidents during the analysis. Table 5 summarizes the caloric value of feces collected from cows which were consuming only grain. The errors are of the same magnitude as those in Table 4. The samples in both Tables 4 and 5 were weighed into the combustion capsules while wet except for the dried pulverized samples. Therefore the caloric values are comparable without considering dry matter. It appears that if losses occurred they were negligible.

Further evidence that losses of energy due to drying were negligible is presented in Table 6 which summarizes 5 experiments in which the feces were burned using dimethyl formamide as the primer. The caloric values of the corresponding dried pulverized samples are also listed for comparison, but the comparison is confounded by dry matter analysis. One of the most striking facts is that there were so many incomplete combustions and accidents which occurred when primers were used. The standard errors of duplicate complete combustions are in the range of 7 to 11.5 cal./g regardless of the source of the sample. The data found in Table 7 shows larger errors for the grain fed animals than for those fed hay. Sampling errors are the most probable explanation, since no primer was added to the capsules which were dried at 80° C. and the errors are also large. It may be noted that the standard error of duplicates of feces weighed wet, dried at 80° C. and burned with dimethyl formamide is almost identical to the error resulting from the combustion of samples treated in the same manner except that no primer was added. This fact, plus the numerous analyses of primers, dried, and wet feces which have standard errors of duplicate determinations of approximately 10 calories per gram, seems to indicate that for precise analysis (less than 0.5% error) it is necessary to use dried samples.

Most of the fecal samples used in these studies had caloric values of approximately 1000 calories per gram of wet material, so a standard error of 10 cal./g would amount to a 1% error. The corresponding samples which were dried and pulverized contain approximately 4560 cal./g, so a standard error of 10 cal./g results in a 0.26% error.

However, if the losses which occur during the drying and pulverizing procedure are appreciable, the loss in analytical precision would be warranted. Some materials, such as silage and possibly some samples of feces, may contain large quantities of combustible volatile compounds which would be lost during the drying process. If the caloric values of these compounds are different from that of the material being analyzed, large errors in the "apparent" gross energy content may occur, as explained by Fenner (1959). Under these circumstances and in those instances where rapid determinations are desired or it is not convenient or advisable to dry the samples, it is advantageous to use primers.

SUMMARY

A series of studies were conducted to determine the suitability of various materials as primers for the wet combustion of high moisture materials, the magnitude of errors associated with gross energy determinations, and the possible losses of energy due to drying bovine feces at different temperatures. The results of 518 analyses of wet feces, 102 analyses of dried pulverized feces, and 119 analyses of primers are presented. There were 85 analyses of wet feces out of the 518 which showed obvious incomplete combustion or accident, while only one of the 102 analyses of dried pulverized samples was questionable. The standard errors of duplicate determinations were calculated for all samples in which complete combustion occurred and the values were approximately 10 calories per gram regardless of the source of the sample or its gross energy content (Table 8). The magnitude of the error resulting depended upon the gross energy content of the sample which was approximately 1000 cal./g for wet feces and 4560 cal./g for dried pulverized feces. Therefore, the precision in wet combustion would be approximately 1% under closely controlled conditions as opposed to 0.26% standard error of duplicates of dried pulverized feces. Losses of energy due to drying were small and were not detectable in most of the trials.

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Table 1. Volatile losses of primers (1 ml. in open dish 5 min.)

Primer	Boiling Point	Room Temperature	Loss	
	°C	°C	mg/min.	cal./min.*
Ethanol	78.5	20.6	2.2	15.4
Ethylene glycol monomethyl ether	124.3	19.8	0.5	2.9
Ethylene glycol monoethyl ether	135.1	20.5	0.1	0.7
Ethylene glycol monobutyl ether	170.6	20.2	0.0	0.0
Dimethyl formamide	198.0	21.7	0.0	0.0

* Calculated from theoretical caloric values.

Table 2. Caloric values of primers

Primer	No. Analyses	Caloric Value		Standard Error of Duplicates*	
		cal/g ± std. dev.		cal/g	%
Dimethyl formamide	73	6290	23	9.9	0.16
Ethylene glycol monobutyl ether	36	7683	20	10.6	0.14
Pyridine	7	8260	40	18.1	0.22
Ethylene glycol monoethyl ether	3	6707	47	--	--

* Calculated from duplicate analyses run on the same day.

Table 3. Gross energy of bovine feces as determined by using various primers and by drying (F32C; ave. 21.38% dry matter)

Treatment	No. Analyses	No. Incomplete Combustions*	Gross Energy Wet Feces	Standard Error of Duplicates	
			cal/g	cal/g	%
Dimethyl formamide	4	1	1016	8.6	0.85
Butyl cellosolve **	4	2	976	2.8	0.29
E.G. monoethyl ether	7	1	1008	6.1	0.61
Pyridine	6	1 (1)	990	19.2	1.94
E.G. monomethyl ether	2	1	1012	--	--
Ethylene glycol	4	3	1007	--	--
Methyl lactate	3	1 (1)	989	--	--
Dried at 97° C	2	0	992	14.5	1.46
Dried, pulverized	4	0	(4716)***	8.3	0.18

* Incomplete combustions plus the obvious accidents, which are in parentheses.
** Butyl cellosolve is ethylene glycol monobutyl ether.
*** All samples except the dried pulverized feces were weighed and calculated on a wet basis. This is equivalent to 984 cal/g wet feces (4716 x 20.88%).
**** Isobutylene glycol and benzoic acid were used but no complete combustions resulted due to poor mixing of primer and feces.

Table 4. Combustion of wet feces by different treatments (Exp. 5 - steers consuming pelleted mixed rations; ave. 25.53% dry matter)

Treatment	No. Analyses	No. Incomplete Combustions	Gross Energy Wet Feces	Standard Error of Duplicates	
			cal/g	cal/g	%
Dimethyl formamide	32	4 (4)	1175	6.1	0.53
Butyl cellosolve	32	1 (4)	1176	14.4	1.22
Dried at 65° C	30	0 (1)	1169	10.8	0.92
Dried at 99° C	30	0	1165	10.0	0.86
Dried, pulverized	24	0	(4500)*	10.0	0.31

* All samples except the dried pulverized feces were weighed and calculated on a wet basis. This is equivalent to 1118 cal/g wet feces.

Table 5. Wet combustion of feces collected from cows receiving only grain (Exp. 3; ave. 20.95% dry matter)

Treatment	No. Analyses	No. Incomplete Combustions	Gross Energy Wet Feces	Standard Error of Duplicates	
			cal/g	cal/g	%
Dimethyl formamide	12	1	906	10.0	1.10
Butyl cellosolve	12	1	928	6.1	0.66
Dried at 99° C	8	1	918	6.4	0.69
Dried, pulverized	12	0	(4393)*	18.1	0.41

* Equivalent to 905 cal/g wet feces.

Table 6. Comparison of errors involved in wet and dry combustions of bovine feces using dimethyl formamide as a primer for wet feces

Treatment	Wet Combustion						Dried Pulverized Feces				
	No. Anal.	No. I.C.	% D.M.	Gross Energy	Std. Error Duplicates		No. Anal.	No. I.C.	Gross Energy	Std. Error Duplicates	
				cal/g	cal/g	%			cal/g	cal/g	%
1. Steers	24	4	24.0	1093 (4561)	10.46	0.96	20	0	4513	10.67	0.24
2. Cows	48	7	22.2	1024 (4624)*	8.98	0.88	22	0	4638	17.09	0.37
3. Cows	46	10	21.5	1001 (4658)	11.49	1.15	8	1	4650	10.74	0.23
4. Cows	24	2	23.3	1079 (4618)	7.59	0.71	12	0	4610	3.66	0.08
5. Cows	16	3	23.2	1061 (4571)	7.02	0.66	-	-	--	--	--

* Dry matter basis for comparison with the dried, pulverized feces gross energy values which correspond to the same samples.

1. Steers-Mixed Ration (Exp. 5B)
2. Cows-All Hay (Exp. 4)
3. Cows-All Hay (Exp. 6)
4. Cows-Pelleted Hay (Exp. 7)
5. Cows-Mixed Rations (Exp. 9)

Table 7. Errors involved in gross energy determinations of wet feces

Treatment	No. Analyses	No. I.C. or Erratic	% D.M.	cal/gm Wet Feces	Standard Error of Duplicates cal/gm	%
Hay Fed Cows (Exp. 10B)						
DMF + Autoclaved Feces	18	3	-	1029	4.02	0.39
DMF + Frozen Feces	18	3	-	1059	15.42	1.46
Dried at 80° C	18	0	-	1051	7.93	0.75
Grain Fed Cows (Exp. 10B)						
DMF + Autoclaved Feces	12	4	-	1049	10.73	1.02
DMF + Frozen Feces	12	5	-	1067	30.57	2.86
Dried at 80° C	12	1	-	1065	20.72	1.95
Cows Fed Mixed Rations (Exp. 10)						
DMF + Autoclaved Feces	20	3	15.867	725	16.29	2.25
DMF + Frozen Feces	20	7	15.766	711	6.88	0.97
DMF + Dried Feces	20	3	15.689	720	9.58	1.33
Dried at 80° C	20	1	15.332	714	9.70	1.36

Table 8. Summary of methods used in the analysis of bovine feces for gross energy

Treatment	No. Analyses	No. Incomplete Combustions or Erratic	Average Gross Energy	Standard Error of Duplicates	
			cal/g	cal/g	%
Wet Feces + DMF	304	49 (24)	1008	11.22	1.11
Wet Feces + DMF 1/	292	46 (22)	1006	10.05	0.999
Wet Feces + B.C.	48	4 (4)	1106	12.11	1.095
Wet Feces + E.C.	7	1	1008	6.11	0.61
Wet Feces + Pyridine	6	1 (1)	990	19.2	1.94
Wet Feces dried prior to combustion	140	1 (6)	996	11.09	1.114
Wet Feces dried prior to combustion 2/	128	1 (5)	989	9.73	0.984
Dried, pulverized feces	102	1	4562	12.04	0.264
Primers only:					
Dimethyl formamide	73	---	6290	9.89	0.16
Butyl cellosolve	36	---	7683	10.64	0.14
Pyridine	7	---	8260	18.11	0.22

1/ Excluding 1 series, Experiment 10B, frozen feces from grain fed cow.
2/ Excluding 1 series, Experiment 10B, feces from grain fed cow.